EFFECT OF HYPOPHYSECTOMY ON THE FUNCTION OF THE KIDNEY OF THE EURYHALINE TELEOST, FUNDULUS KANSAE[1]

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It now seems well established that hypophysectomy markedly reduces the ability of several euryhaline teleosts to survive in fresh water (Pickford *et al.*, 1965; Ball and Ensor, 1965; Handin *et al.*, 1964; Schreibman and Kallman, 1963, 1964; Ball, 1962; Pickford and Phillips, 1959; Pickford and Atz, 1957). The available data also suggest that such fishes are unable to maintain normal levels of blood-electrolytes in such an environment (Ball and Ensor, 1965; Leloup-Hatey, 1964; Burden, 1956), which leads to the supposition that the function of those organs most intimately concerned with salt and water metabolism, *i.e.*, the gills and kidney, has been affected by hypophysectomy. To date, few data have appeared to support such a supposition (Stanley and Fleming, 1963; Chester Jones *et al.*, 1965).

The renal physiology of the euryhaline teleost, *Fundulus kansae*, has been a subject of investigation in this laboratory for some time, and, during the course of these studies, several effects of hypophysectomy have been observed. We wish here to report the results of such studies.

Materials and Methods

Fundulus kansae was collected from a salt spring "Boonslick" located in Howard County, Missouri. The routine handling of the animals, the methods used to obtain urine and serum samples, to estimate urine volumes and glomerular filtration rates (GFR), and the analytical methods employed have all been described elsewhere (Fleming *et al.*, 1964; Stanley and Fleming, 1964; Fleming and Stanley, 1965) and need not be repeated here. Hypophysectomy was carried out *via* the opercular route recommended by Abramowitz (1937) under MS-222 (Sandoz) anesthesia. Controls were sham-operated in the same manner, except that the bone covering the pituitary was not pierced. With one exception noted below, the operation was performed on fresh-water-adapted fish which were returned to fresh water. Survival after hypophysectomy is good, providing that adequate calcium ion is made available to the animals (unpublished data).

The sea water used here was prepared from a commercial salt mix (Seven Seas Marine Mix, Utility Chemical Co., Patterson, New Jersey), and was brought to a concentration of 1000 mOsm/kg. All experiments were run at 19 ± 10° C.

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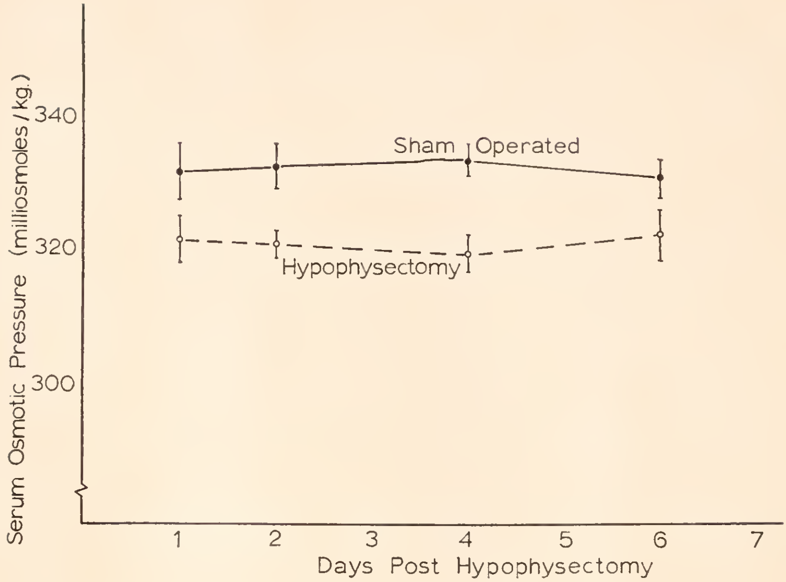

FIGURE 1. Effect of hypophysectomy on the serum osmotic pressure of the killifish, *F. kansae*, held in fresh water. Data show the mean ± S.E. for 7 animals at each point.

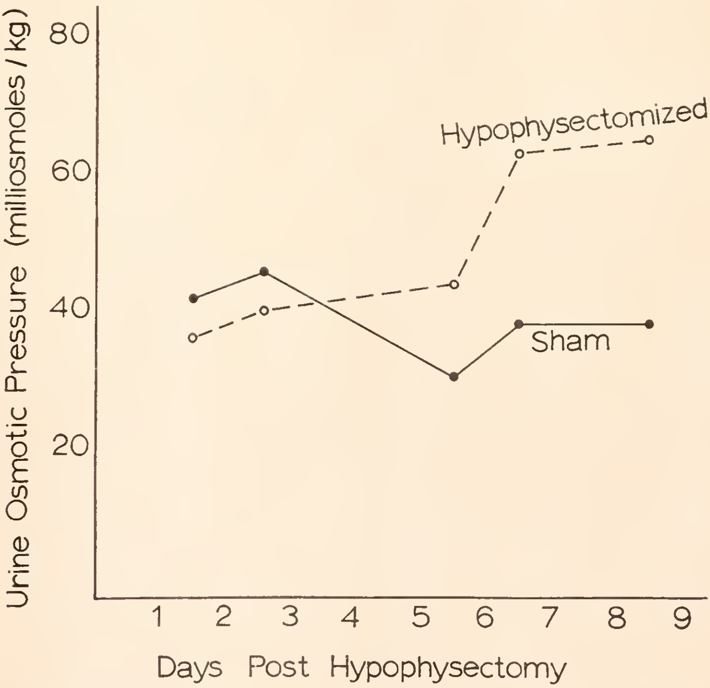

FIGURE 2. Effect of hypophysectomy on the osmotic pressure of the urine of animals in fresh water. Each point is the mean taken from three animals.

Results

1. *Time sequence of responses to hypophysectomy*

The responses to hypophysectomy reported here do not appear simultaneously. As shown in Figure 1, a drop of the osmotic pressure of the serum of hypophysectomized fish is observed on the day after surgery. Since this response is seen only in hypophysectomized fish and is maintained indefinitely, it cannot be attributed to shock alone. In another experiment the serum osmotic pressure was 330 ± 3.6 milliosmoles (mean ± S.E.) for sham-operated animals and 312 ± 1.8 milliosmoles for hypophysectomized animals at 13 days post-operation.

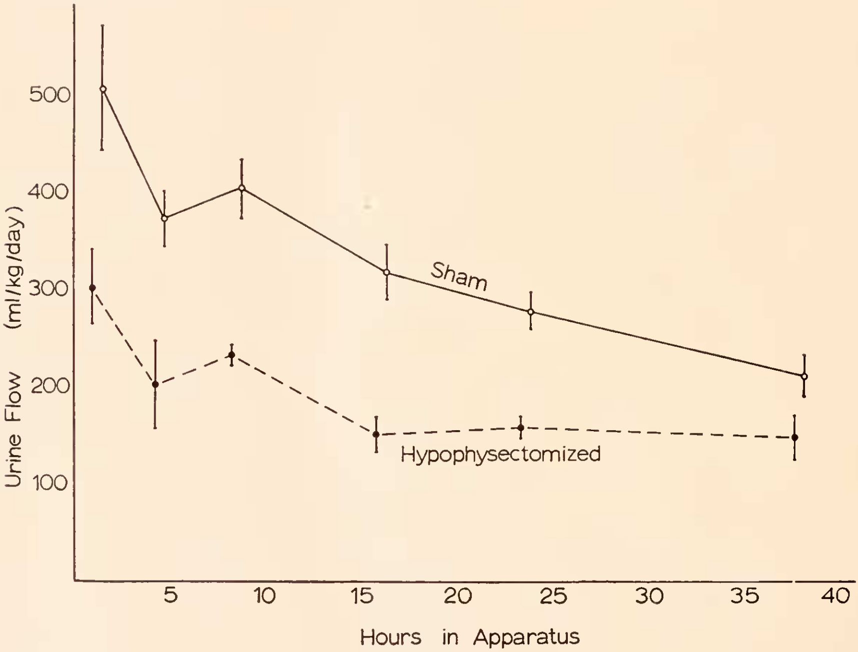

FIGURE 3. Comparison of urine flow of sham-operated and hypophysectomized *F. kansae* held in fresh water. Data show the mean flow ± S.E. for 7 animals.

A second response, a rise of the osmotic pressure of the urine (Fig. 2), does not appear until approximately one week after hypophysectomy. The appearance of still a third response, *i.e.*, a drop in the rate of urine excretion (Fig. 3), is somewhat variable, and may or may not be present at the time that the osmotic pressure of the urine starts to rise. It has been our practice to hold hypophysectomized animals for 12–14 days before use, in order to ensure that the above responses will all be present. As shown in Table I, the changes in the osmotic pressure of the urine and serum reflect changes in the concentration of the electrolytes found in these fluids. Urine sodium is elevated markedly, and urine potassium to a lesser extent. At the same time, serum sodium levels are reduced slightly. Total-body sodium is also less than that of the controls. No changes were noted in serum or total-body

TABLE I

Effect of hypophysectomy on urine and serum sodium and potassium, total body sodium and potassium, and total body water. The values given are the mean ± S.E. The number of animals in each sample is given in brackets. Urine data based on samples collected between the 21st and 26th hours after cannulation

	Sodium (meq/l.)	Potassium (meq/l.)	Water content (%)
Urine			
Hypophysectomized	47.8 ± 7.6 (11)	1.06 ± 0.20 (11)	
Sham-operated	15.1 ± 1.1 (10)*	0.45 ± 0.10 (10)*	
Serum			
Hypophysectomized	145.9 ± 2.5 (9)	4.17 ± 0.42 (11)	
Sham-operated	157.8 ± 1.3 (13)*	3.80 ± 0.29 (11)	
Total Body	(meq/kg.)	(meq/kg.)	
Hypophysectomized	55.2 ± 2.1 (18)	64.8 ± 1.02 (12)	74.88 ± 0.34 (12)
Sham-operated	62.5 ± 1.0 (12)*	65.4 ± 1.63 (18)	74.62 ± 0.29 (12)

* $P = 0.05$.

potassium levels, or in the percentage of body water. The observed reduction in urine flow may serve to reduce renal sodium loss somewhat but is not entirely effective. Thus, as shown in Table II, hypophysectomized animals lost, over a 45-hour period, over twice as much sodium *via* the kidney as did their controls.

2. *The diuretic response*

An examination of Figures 3, 5, and 6 shows a characteristic response of control animals to handling, *i.e.*, a marked diuresis of variable duration. Examination of the data for the controls reported in Figure 4 indicates that the diuretic phase is due, in part, to reduced tubular reabsorption of water, *i.e.*, the urine/serum C^{14} inulin ratio is less than 2 for several hours after cannulation. In other experiments, we have measured U/S ratios close to unity during the diuretic peak. Figure 5 shows that the diuretic response of the controls is also due, in part, to an elevated GFR. The same data show that while hypophysectomized animals may show some initial diuresis, the response is markedly reduced. The data re-

TABLE II

Renal sodium loss of hypophysectomized and control animals over a 45-hour period. Each value is the mean ± S.E. measured from a sample of 7 animals

μeq. Urinary Na^+/gm./hr.		
Hours	Hypophysectomized	Controls
0–3	0.99 ± 0.11	0.70 ± 0.12
3–11	0.79 ± 0.11	0.36 ± 0.06
11–21	0.49 ± 0.15	0.16 ± 0.01
21–31	0.56 ± 0.12	0.20 ± 0.03
31–45	0.53 ± 0.09	0.16 ± 0.02
Total Na^+ loss	27.21 ± 2.75	10.82 ± 0.50

ported in Figure 4 indicate that hypophysectomized fish do not reduce tubular reabsorption of water during the diuretic period. However, hypophysectomized animals also do not show an elevated GFR as seen in controls during the diuretic period (Fig. 5).

3. *Effects of a sudden change of environment*

Hypophysectomy also affects the renal responses shown by *F. kansae* when transferred suddenly from a hypotonic to a hypertonic environment. Figure 6 compares the rates of urine flow of hypophysectomized and control animals that

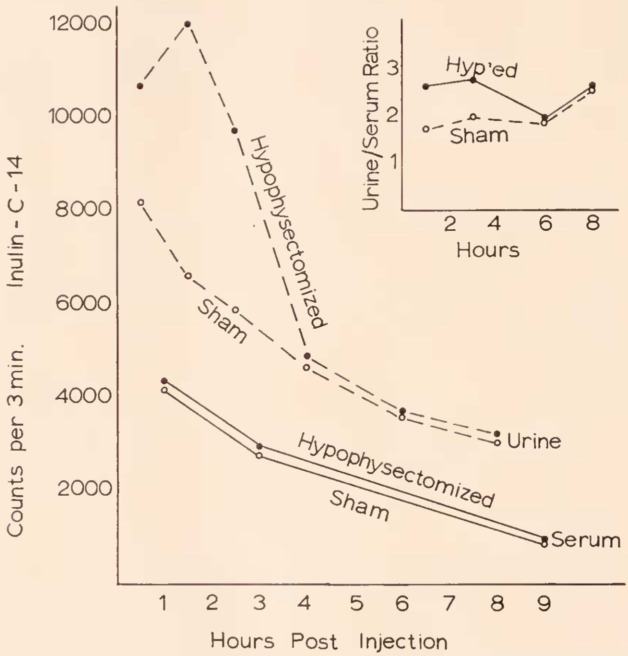

FIGURE 4. Rate of clearance of C^{14} inulin from the serum and its appearance in the urine of fish in fresh water. Serum and urine data at each point are the means of three determinations.

were switched suddenly from fresh water to sea water. Examination of Figure 6 shows that the control animals were able to reduce urine flow rapidly, and had established a new and steady rate of urine flow after being in sea water for 5 or 6 hours. Hypophysectomized animals also reduce urine flow, but do so at a much slower rate. Thus, in the experiment reported in Figure 6, these fish required almost 40 hours to reach levels that their controls had achieved by four hours post-transfer.

Figure 7 reports the changes in the osmotic pressure of the urine measured from the same experiment. Inspection of Figure 7 shows that the osmotic pressure of the urine taken from hypophysectomized animals in fresh water was almost triple that of their controls. However, the situation was quickly reversed after

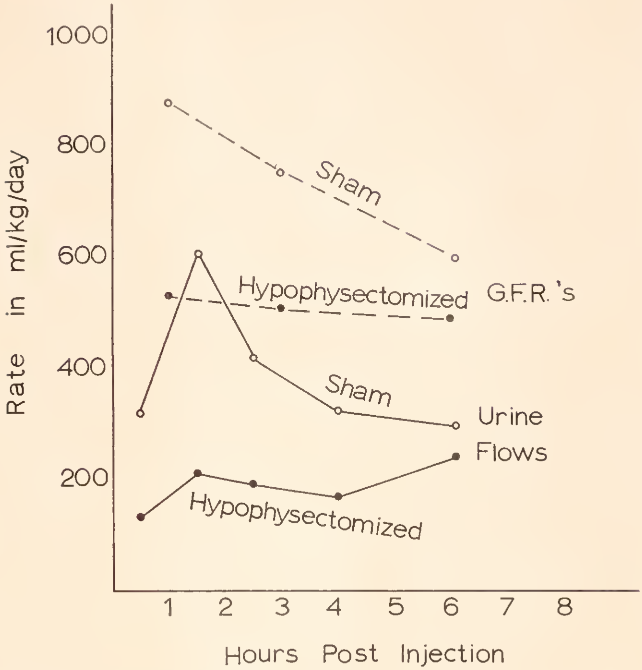

FIGURE 5. Glomerular filtration rates of sham-operated and hypophysectomized *F. kansae* in fresh water. GFR's calculated from the data in Figure 4 and the urine flow.

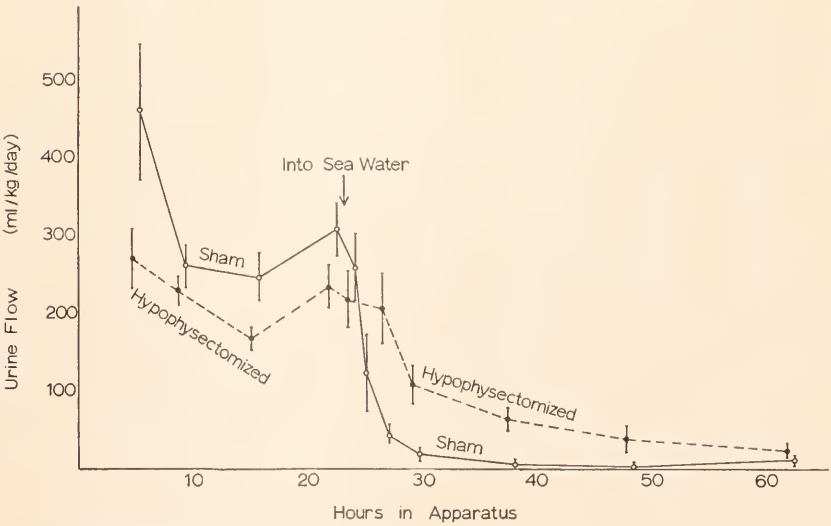

FIGURE 6. Effect of a sudden transfer from fresh water to sea water on urine production. Each point shows mean ± S.E. for 7 animals.

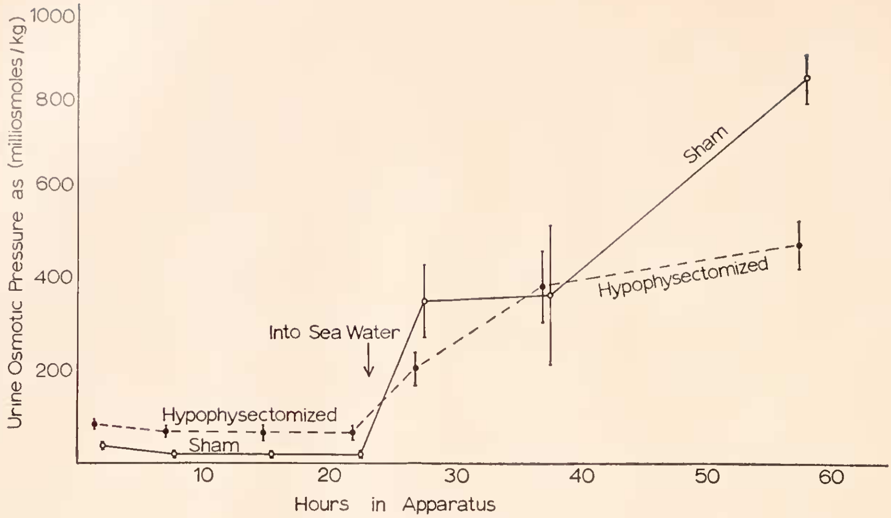

FIGURE 7. Changes in the osmotic pressure of the urine which follow transfer from fresh water to sea water. Data collected from the same experiment shown in Figure 6.

switching the environment to sea water. Further, as shown in Table III, the osmotic pressure of urine taken from the control animals at the termination of the experiment was hypertonic to the serum, while that of the hypophysectomized animals was hypotonic. The ability of *F. kansae* to excrete a blood-hypertonic urine has been reported previously (Stanley and Fleming, 1964; Fleming and Stanley, 1965).

4. *Pre-adaptation and serum osmotic pressure*

During the course of other experiments, it was noted that a short exposure to saline would markedly alter the course of adaptation to sea water, at least as judged by the magnitude of the changes in the osmotic pressure of the serum which follow a transfer from fresh water to sea water. Figure 8 reports the effect of a 9-hour exposure to 40% sea water on the changes in serum osmotic pressure

TABLE III

Comparison of the osmotic pressure of the serum and urine of control and hypophysectomized animals. Data taken at the termination of the experiment shown in Figures 6 and 7. The lower number of urine samples is due to the failure of some animals to excrete sufficient urine for a measurement. Osmotic pressures in mOsm./kg.

	Control			Hypophysectomized		
	N	$\bar{x}$	±S.E.	N	$\bar{x}$	±S.E.
Serum	5	326	11	6	313	20
Urine	3	455	27	4	256	26

which followed such a transfer. In this experiment the routine followed was 9 hours in 40% sea water, 18 hours in fresh water, and then a transfer into 100% sea water. As shown in Figure 8, 18 hours in fresh water was sufficient time for the osmotic pressure of the serum to fall to levels identical to that of controls that had been held in fresh water continuously. Figure 8 also shows that the short exposure to dilute sea water markedly changed the response to the challenge presented by full-strength sea water.

As shown in Figure 9, hypophysectomy alters the response seen when preadapted animals are transferred from one environment to the other. In this

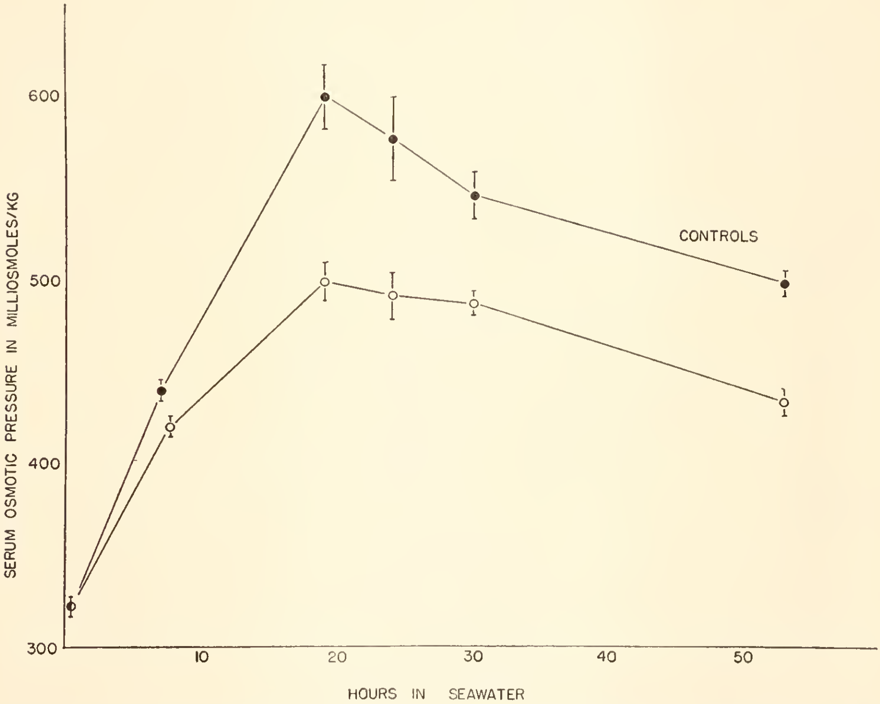

FIGURE 8. The effect of a 9-hour exposure to 40% sea water on the ability of *F. kansae* to adjust to a transfer from fresh water to sea water. Each point shows the mean ± S.E. for 7 animals.

experiment, a large number of fish (160) were held in sea water for several weeks. After this adjustment period, half the animals were hypophysectomized and returned to sea water. Five days later all the fish were transferred to fresh water for 48 hours, sufficient time for the osmotic pressure of the serum of the hypophysectomized fish to fall below that of their controls. The fish were then transferred back into sea water and the osmotic pressure of the serum followed for 23 hours. Then the remaining fish were returned to fresh water. Figure 9 shows that the serum osmotic pressure of the hypophysectomized fish rose above that of their controls after the first transfer, and, for that matter, appeared to be rising slowly long after the controls had reached a fairly steady plateau. The hypophysectomized animals do show some signs of their past history, however, for the peak values reached were still well below those shown by still another control group which had not been exposed to saline before being transferred into sea water.

Neither group had any difficulty in readjusting to fresh water, although, once again, the time course and degree of adjustment was somewhat different in the two groups. Thus, by $8\frac{1}{2}$ hours after the return to fresh water, the serum osmotic pressure of the control animals was significantly lower ($P = 0.05$) than that measured at the start of the experiment. Then the osmotic pressure rose to the original levels. The serum osmotic pressure of the hypophysectomized animals

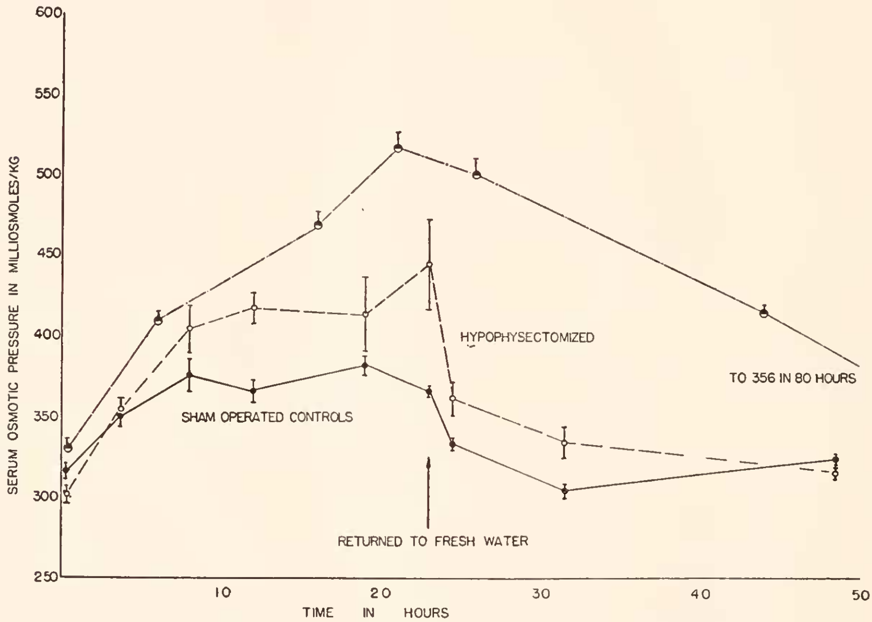

FIGURE 9. Effect of hypophysectomy and pre-adaptation to sea water on the ability of *F. kansae* to adjust to a sudden transfer from fresh water to sea water. The hypophysectomized animals and their controls had been adapted to sea water for several weeks and then returned to fresh water for 48 hours. The other group of controls (top line) had not been exposed to saline. Each point shows the mean ± S.E. for 6 animals.

dropped precipitately immediately after the return to fresh water ($1\frac{1}{2}$ hours) and then fell slowly towards the original values, although these had not been reached at the time the experiment was terminated.

DISCUSSION

It is clear that *F. kansae* can survive in fresh water after hypophysectomy. In this regard the plains killifish more closely resembles the European eel (*Anguilla anguilla* L.) which also survives well after such surgery (Fontaine *et al.,* 1949; Chester Jones *et al.,* 1965), than it does a number of other euryhaline teleosts including the closely related *F. heteroclitus* (Pickford *et al.,* 1965). There are, however, clear-cut differences between this killifish and the eel, for the latter survives well in distilled water—even after hypophysectomy (Chester Jones *et al.,* 1965). *F. kansae,* on the other hand, will not tolerate such an environment for more than a few days, at best.

While the plains killifish can survive in fresh water after hypophysectomy, removal of the pituitary does alter the osmoregulatory patterns of the animal. The kidney is one of the target organs affected, as shown by a drop in volume and a rise in osmotic pressure of the excreted urine. A preliminary report (Chester Jones *et al.,* 1965) indicates that the eel shows a similar response for urine volumes, but in this case, there was a reduction in urinary sodium loss.

Since *F. kansae* survives well in fresh water after hypophysectomy, it seems clear that the accelerated loss of sodium *via* the kidney does not present an insurmountable challenge to the animals. Nevertheless, the changes seen are not trivial and raise a number of questions. Thus it becomes useful to learn if the reduction in urine flow is due to (1) a reduction in GFR, or (2) an increase in the tubular reabsorption of water.

On the surface, at least, the first alternative would seem to offer the simplest explanation of our data, and would only require as a corollary that the tubular reabsorption of ions be reduced after hypophysectomy. A large body of literature exists to provide precedent for such responses.

There are, however, a number of difficulties. Thus, if one considers that a reduction of GFR has followed hypophysectomy, it then becomes necessary to suppose that these animals must either evoke another route of water excretion in order to handle the ever-impinging water load, or effectively reduce the net influx of water across permeable surfaces.

A second difficulty lies in the report of Holmes and McBean (1963), who found that corticosterone markedly reduced the GFR of rainbow trout (*Salmo gairdneri*) held in fresh water. The implication is that glomerular filtration rates are, at least in part, under the control of the pituitary-interrenal axis. Our results are not inconsistent with such an implication, but would require that such control would serve to increase the GFR, rather than to reduce it. Unfortunately, Holmes and McBean (1963) did not use hypophysectomized animals, and the dose of steroid administered (5 mg.) seems to be beyond the physiological range.

A third difficulty lies in the fact that, to date, we have not been able to show any difference between the filtration rates of hypophysectomized and sham-operated controls, except during the diuresis which follows handling. This point is still under investigation and must remain open.

The second alternative, *i.e.*, an increase in the tubular reabsorption of water, differs from the first in that it does not require as marked a change in the pattern of ion reabsorption. It would seem to require, however, an even more drastic solution to the problem of the impinging water load, for an increase in water reabsorption would hardly be a positive contribution to the necessity of eliminating excess water—unless one supposes that water permeability is controlled by the rate of urine excretion, instead of *vice versa.* A decision between the two alternatives (if indeed the situation is that simple) cannot be made until the question of filtration rates is closed.

It may well be that the situation is not so simple, and, in this regard, Chester Jones *et al.* (1965) have recently put forward a hypothesis that would, if demonstrated, complicate the situation considerably. They suggest that in the eel, urine has a double original, *i.e.,* from the glomerular filtrate and from the addition of water to the filtrate *via* the peritubular capillaries of the renal portal system. This hypothesis was advanced to explain the paradoxical effects of hormones on the renal function of the eel. We have not considered the possibility of such a mechanism operating in *F. kansae* nor have we studied the effects of neurohypophysial hormones on renal function, but we can point out that mammalian ACTH does not affect the rate of urine excretion of hypophysectomized killifish (Stanley and Fleming, 1963).

It is well known that teleosts are slow to show responses to hypophysectomy, at least as judged by histological criteria (Handin *et al.,* 1964; Pickford and Atz, 1957). Our data show that some physiological responses appear in a relatively short period after surgery, a result also found by Ball and Ensor (1965) and Chester Jones *et al.* (1965). Thus it appears that pituitary control may, in some cases at least, be more precise than that suggested from histological studies.

The data presented in Figure 9 suggest that the pituitary may be more involved in the adjustments required of *F. kansae* when presented with a blood-hypertonic challenge than when the opposite situation obtains. Such results are consistent with a number of studies which demonstrate that the interrenals often show signs of hyperactivity when such transfers are carried out (Olivereau, 1962).

Summary

Hypophysectomy has been shown to affect the pattern of urine formation and excretion by the euryhaline killifish, *Fundulus kansae.* Changes noted in fresh water include (a) a drop in urine volume, (b) a rise in osmotic pressure, due in part to increased concentrations of sodium and potassium in the urine, and (c) a marked increase in the time required to shut off urine flow when transferred suddenly from fresh water to sea water. Hypophysectomized animals in fresh water also show a slight but consistent reduction in the osmotic pressure of the serum. Pre-exposure to saline increases the ability of hypophysectomized animals to respond to an osmotic challenge, but they do not respond as well as their controls.

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